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**Citation for published version:**

Riggio, V & Portolano, B 2015, 'Genetic selection for reduced somatic cell counts in sheep milk: A review', *Small Ruminant Research*, vol. 126, no. Supplement 1, pp. 33-42.  
<https://doi.org/10.1016/j.smallrumres.2015.01.020>

**Digital Object Identifier (DOI):**

[10.1016/j.smallrumres.2015.01.020](https://doi.org/10.1016/j.smallrumres.2015.01.020)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Small Ruminant Research

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**Genetic selection for reduced somatic cell counts in sheep milk: A review**

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## ABSTRACT

Mastitis is an inflammation of the udder, mainly caused by bacteria, and leads to economic loss, due to discarded milk, reduced milk production, reduced milk quality, and increased health costs in both dairy sheep and cattle. Selecting for increased genetic resistance to mastitis can be done directly or indirectly, with the indirect selection corresponding to a prediction of the bacteriological status of the udder based on traits related to the infection. The most frequently used indirect method is currently milk somatic cell count (SCC) or somatic cell score (SCS). This review reports the state of the art relating to the genetic basis of mastitis resistance in sheep, and explores the opportunities to use SCC as selection criterion in a breeding programme to improve resistance to mastitis in sheep, discussing the actual situation and prospects for improvement. It has been stressed, in particular, that although it is unlikely that selection for mastitis resistance by the farmers on their own will be successful, there is good prospect for genetic improvement if reliable pedigree and performance recording is implemented across flocks, combined with breeding value estimation. To achieve this, a strong and well-structured organization to implement and support the program is essential.

**Key words:** mastitis, genetic selection, somatic cell count, sheep

## 1. Introduction

The Mediterranean Basin countries host 60% of the total world sheep and goat milk production. The dairy sheep and goat industry is usually based on local breeds, which are very well adapted to the production systems and environments. Milk production is the principal trait affecting the profitability of these industries, and therefore for long time the breeding programmes have considered milk production as the major selection criterion.

However, due to the EU agricultural policy and consumer demands, increased attention has been focused on traits related to the reduction of production costs, food safety and health (e.g. resistance to intramammary infections, internal parasites, scrapie, etc.). Mastitis, in particular, is one of the main infectious diseases in dairy sheep and goats as well as in dairy cattle – with respect to dairy industry and public concern, economic impact, zoonotic potential and animal welfare (Davies et al., 2009).

This review reports the state of the art relating to the genetic basis of mastitis resistance in sheep, and explores the opportunities to use somatic cell count (SCC) as a selection criterion in a breeding programme to improve the resistance to mastitis in sheep, discussing the actual situation and prospects for improvement.

## **2. Mastitis and mastitis-causing pathogens**

Mastitis is an inflammation of the udder and it leads to economic loss, mainly due to discarded milk, reduced milk production and quality and increased health costs (Miller et al., 1993; Allore and Erb, 1998; Leitner et al., 2003). Rupp and Foucras (2010) reported that the total annual milk production losses due to mastitis in small dairy ruminants can be estimated to be in the region of €60 million/annum.

Mastitis can be classified as subclinical or clinical. Mastitis is subclinical when no visible changes occur in the appearance of both the milk and udder, but milk production decreases, bacteria are present in milk and the milk composition is altered (Harmon, 1994). On the other hand, mastitis is clinical when symptoms such as fever, abnormal texture and discoloration of the milk, increased temperature or pain of the quarter or udder half, and a change in milk properties occur. Generally, the incidence of clinical mastitis in cattle varies between 20 and 40% per cow/year (Heringstad et al., 2000). In small ruminants, the annual incidence of

65 clinical mastitis is generally lower than 5% (Bergonier and Berthelot, 2003; Contreras et al.,  
66 2007), whereas the incidence of subclinical mastitis in these species has been estimated at 5-  
67 30% per lactation or even higher (Bergonier and Berthelot, 2003; Contreras et al., 2003).

68 Mastitis-causing pathogens include bacteria and non-bacterial pathogens, like mycoplasmas,  
69 fungi, or viruses (Bergonier and Berthelot, 2003). Among viruses, the Maedi-Visna virus is  
70 one of the main causes in sheep, having being associated to mastitis, as well as chronic  
71 inflammatory lesions in the lungs, joints, and brain (Radostits et al., 2007). However, given  
72 that the occurrence of non-bacterial pathogens is far less frequent, they will not be further  
73 considered in this review.

74 The bacterial pathogens responsible for infection of the mammary gland (in particular  
75 coliform bacteria, staphylococci and streptococci) may be split into two main categories,  
76 according to the severity of the clinical signs, namely major and minor pathogens. Infection  
77 with major pathogens generally results in clinical illness or strong inflammatory responses  
78 and reduced milk yields, whereas minor pathogen infection is usually subclinical (White et  
79 al., 2001). Pathogens can also be categorised, depending on their aetiology, into  
80 environmental or contagious (Fox and Gay, 1993):

81 i) Environmental bacteria (found in the soil, faeces, and bedding), which enter the teat duct  
82 from these sources and include both Gram-positive and Gram-negative bacteria such as  
83 *Streptococcus non-agalactiae* and coliform organisms (*Escherichia coli*, *Klebsiella* sp.,  
84 *Aerobacter aerogenes*, *Enterobacter* sp.);

85 ii) Contagious bacteria, which are transmitted from infected quarters or halves to non-  
86 infected quarters or halves during the milking process and include such Gram-positive  
87 bacteria as *Staphylococcus aureus* and *Streptococcus agalactiae*.

In cattle, coagulase-negative staphylococci (CNS) are considered to be minor pathogens; this, however, is less clear in sheep, in which CNS are considered the most common bacterial species causing both subclinical and clinical mastitis (Albizu et al., 1991; Amorena et al., 1991; Marco et al., 1991). In chronic cases, Gonzalo et al. (1998) suggested dividing the CNS into two groups with different pathogenicity between dairy sheep: NRCNS (novobiocin-resistant CNS), which behave as minor pathogens, resulting in mild changes in SCC and milk yield and similar to those commonly associated with micrococci and Corynebacteria (Ziluaga et al., 1998). Also NSCNS (novobiocin-sensitive CNS), which cause more substantial changes in SCC and loss in milk yield, similar to those associated with the classic major pathogens (Peris et al., 1996).

### **3. Selection criteria to select for mastitis resistance**

Mastitis resistance is a complex trait, involving both genetic and environmental factors, including infection pressure. In the broadest sense, resistance could be defined as the ability to avoid any infection and/or the quick recovery from an infection (Rupp and Boichard, 2003). It involves different components, namely avoiding entry of the pathogen into the mammary gland, mounting an immune response capable of limiting its development in the udder and clearing the infection, as well as controlling the pathogenic effects of the infection, such as, e.g., tissue damage (Rupp and Foucras, 2010).

Selecting for increased genetic resistance to mastitis can be done directly or indirectly. Direct selection relates to the diagnosis of the infection. The actual trait (e.g. bacteriological examination of milk and/or observation of clinical cases of mastitis) is measured on the animal or its relatives. Indirect selection relates to a prediction of the bacteriological status of the udder, based on traits related to the infection (e.g. inflammatory parameters). In this case,

an indicator trait for mastitis is measured on the animal itself or its relatives (de Haas, 2003). A direct bacteriological assay is the recommended method of diagnosis of mastitis (González-Rodríguez and Cármenes, 1996), as it is believed to provide precise and exhaustive information on infected quarters and/or halves and the pathogens involved. However, it is rarely used for genetic purposes, because it is difficult to implement on a large scale. It also has limitations because of the requirement of intensive labour, the time delays for culture to occur, and the costs involved with bacteriology (McDougall et al., 2001). Moreover, it has been shown that bacterial shedding is variable and levels may sometimes be too low to be detected by conventional techniques (Rupp and Foucras, 2010). Therefore, although the bacteriological examination is often considered to be the ‘golden standard’ for routine detection and identification of mastitis pathogens, it has to be taken into account that even good quality bacteriological data will have true sensitivity and specificity values somewhat less than one, i.e. some cases will be missed and others will be misdiagnosed as infected when they are not (Riggio et al., 2010).

Simple, indirect methods have been widely applied, based on the evaluation of the degree of inflammation or of internal mammary lesions (De la Cruz et al., 1994). Their accuracy is usually established by bacteriological analysis as a reference method. Among these methods, the most frequently used to detect mastitis is SCC.

#### **4. Biological signification of SCC**

Somatic cells normally occur in milk of both cattle and small ruminants. Somatic cells consist of many types of cells, including polymorphonuclear leukocytes (PMN), macrophages, lymphocytes, eosinophils, and various epithelial cells from the mammary gland. Cells in milk from a healthy udder are mainly represented by mammary gland epithelium and drain canal

cells. Recently, Leitner et al. (2012) showed that epithelial cells accounted for ~50% of the cells in goats and cows, whereas in sheep this was ~80%. These researchers suggested that sheep shed more epithelial cells into milk in comparison to cows and goats, probably because these cells play an important role in the immune response. According to Walawski (1999) only 8% of the cells are leukocytes and less than 1% are macrophages in cattle. However, in a more recent study Leitner et al. (2012) showed that in bacterial free animals at midlactation, goats had the highest number of leukocytes and PMN. Sheep, on the other hand, had the lowest and cows were intermediate between sheep and goats. It has also been reported that PMN are the major cell population during early inflammation and play a protective role against infectious diseases in the mammary gland (Kehrli and Shuster, 1994; Persson-Waller et al., 1997). Experimental intramammary infection of sheep with *Staphylococcus aureus* or *Escherichia coli* has been shown to induce a significant increase in PMN within 24 h of infection (Persson-Waller et al., 1997).

Determination of the differential cell count in milk is another useful approach to evaluate the proportion of leukocytes during inflammation and thus the immune status of the mammary gland. In ewe milk samples, flow cytometry was used to detect the percentage of PMN, macrophages, and lymphocytes in bulk and individual milk with different concentrations of somatic cells (Albenzio et al., 2009; Albenzio and Caroprese, 2011; Albenzio et al., 2011).

The concentration of somatic cells in milk is defined as SCC and it is expressed as thousands of cells per millilitre of milk. The measure of SCC has the following properties:

- it can be routinely recorded in most milk recording systems;

- the heritability of SCC is higher than the heritability of the direct trait (i.e., mastitis incidence);

- it is usually an indicator of both clinical and subclinical infections.



What is reported thus far shows why SCC is usually considered as a good predictor of mastitis occurrence (milk SCC reflects the number of neutrophils migrating from blood to the mammary gland in response to infection). However, numerous factors influence the SCC level of both infected and non-infected animals, such as the physiological status of the host, the infection status and the pathogen. It is, therefore, difficult to interpret single measures and define fixed thresholds, as distributions of the SCC of infected and non-infected animals overlap considerably (Riggio et al., 2010; Rupp and Foucras, 2010). This aspect will be further analysed in the next sections. From these considerations, it follows that repeated measures or lactation average are usually preferred for both diagnosis and genetic purposes.

The distribution of SCC is positively skewed; whereas, conventional statistical methods usually accommodate normally distributed data. In order to obtain a distribution which closely resembles a normal distribution, the SCC is log-transformed to somatic cell score (SCS). The formula commonly used is:  $SCS = \log_2(SCC/100) + 3$  (Ali and Shook, 1980). However other researchers have used either  $\log_e$  or  $\log_{10}$  logarithmic transformation (Samoré, 2003).

#### ***4.1. SCC in sheep***

While cattle SCC values between 250 and  $300 \times 10^3$  cells/mL are reported as most satisfactory discrimination thresholds between healthy and infected udders, sheep do not have a widely accepted threshold. Some evidence has been provided that healthy ewes have normally higher SCC than cows (Maisi et al., 1987; Fthenakis et al., 1991; González-Rodríguez et al., 1995). Bufano et al. (1996) showed that a high SSC ( $>1$  million/mL) occurs in healthy sheep and goat milk, especially towards the end of lactation. While Riggio et al. (2010) reported that the

183 SCC can be high, even when ewes are not infected, suggesting that a healthy animal can  
184 wrongly be diagnosed as infected based on SCC.

185 On the other hand, considering subclinical mastitis, Leitner et al. (2008) suggested that, while  
186 in dairy cows subclinical mastitis is largely ignored, because the increase in SCC in infected  
187 glands is modest (about  $300\text{-}500 \times 10^3$  cells/mL) and the mixing with the milk from non-  
188 infected quarters is sufficient in most cases to appreciably lower the effect of SCC at the cow  
189 level. In sheep and goats, which have only two mammary glands, mixing of milk with high  
190 SCC coming from an infected gland with a low SCC from a healthy gland might be  
191 insufficient to reduce the SCC at the animal level. However, whether these high SCC are a  
192 consequence of the fairly generalized lack of preventive management measures against  
193 subclinical mastitis in sheep flocks or whether a higher cell discrimination threshold is  
194 required for sheep milk, has not been established.

195 It is important to highlight, however, that the choice of a threshold in the cattle industry was  
196 mostly driven by monetary factors. While little knowledge has been available on the  
197 significance of other factors in keeping farmers motivated to improve mastitis management  
198 (Valeeva et al., 2007). In sheep, some studies reported that similar payment systems (e.g.  
199 reduced milk prices, if the SCC of the bulk tank milk exceeds certain thresholds) are  
200 becoming common (Legarra et al., 2007; Pirisi et al., 2007). However, the current milk  
201 payment system of most breeds and countries is still based only on milk yield and not on  
202 SCC level. This makes it more difficult to choose a threshold to discriminate between healthy  
203 and infected udders, which can be worldwide accepted. Some researchers (Fthenakis et al.,  
204 1991; Jones, 1991) reported discrimination values between healthy and infected glands  
205 ranging from 500 to  $1600 \times 10^3$  cells/mL, while others (Bergonier et al., 1994; De la Cruz et  
206 al., 1994; Pengov, 2001) reported values similar to those for cows ( $200$  to  $300 \times 10^3$  cells/mL).

González-Rodríguez et al. (1995) suggested that breed differences in SCC do exist. Considering several breeds, these researchers reported the value of  $300 \times 10^3$  cells/mL as the most suitable threshold of discrimination for total SCC data. However, within each breed the most suitable threshold was  $400 \times 10^3$  cell/mL for Assaf and Castellana and  $200 \times 10^3$  cell/mL for the Churra sheep breeds.

Recently, it was also suggested that SCC diagnostic effectiveness (SCC ability to detect whether or not intramammary infections occur) may be assessed to a degree without having to commit to a single threshold with the use of average indices based on Receiver-Operating Characteristic (ROC) curves (Riggio et al., 2013). These researchers identified different optimal SCS thresholds, ranging from 2.81 to 3.33, depending on the trait definition (e.g. SCS for the whole sample, SCS for samples with minor pathogen infections, and SCS for samples with major pathogen infections). It was suggested that different SCC (and therefore SCS) thresholds should be used when considering mastitis caused by minor or major pathogens.

## **5. Genetic parameters of SCC and mastitis and correlations with other traits in sheep**

### ***5.1. Genetic parameters of SCC and mastitis in sheep***

Genetic studies of SCC in dairy sheep are more recent and less frequent than in dairy cattle. Heritability estimates, based on repeatability test-day models, range from 0.04 to 0.16 for several breeds including the Churra (Baro et al., 1994; El-Saied et al., 1998; Othmane et al., 2002), the Manchega (Serrano et al., 2003), the East Friesian (Hamann et al., 2004) and the Valle del Belice sheep breeds (Riggio et al., 2007). Other studies reported similar or slightly higher heritability estimates (from 0.11 to 0.18) for the average SCS during lactation, for Chios (Mavrogenis et al., 1999), Lacaune (Barillet et al., 2001; Rupp et al., 2003a), Latxa

(Legarra and Ugarte, 2005) and Manech Red Faced ewes (Barillet et al., 2008). These heritability estimates are comparable to those reported in literature for cattle either with test-day (Carnier et al., 1997; Mrode et al., 1998) or lactation models (Rupp and Boichard, 1999). Moreover, in cattle it has been shown that heritability estimates for SCS are usually higher than heritability for the direct trait (i.e. mastitis incidence). Therefore, when only considering the heritability, these results suggest that selection for SCS (as indicator of mastitis) has to be preferred over selection for the direct trait. However, before conclusions can be drawn, correlations between traits should be considered.

In cattle, for example, genetic correlations between SCS and the incidence of clinical mastitis vary from moderate to high, with an average of approximately 0.7 (Rupp and Foucras, 2010). These results, therefore, confirm that, although SCS and mastitis are not the same trait, SCS can be used as a selection criterion in a breeding programme for mastitis resistance in cattle. In sheep, however, no estimates of genetic correlations between SCC and clinical and subclinical mastitis incidence have been reported in the literature.

On the other hand, when considering data on intramammary infections assessed by bacteriological analyses, only few results are found in the literature. Published studies refer more directly and exhaustively to udder health status. In cattle, heritabilities for intramammary infections varied from 0.02 to 0.04 as reported by Weller et al. (1992). Somewhat higher (0.10 to 0.20) as quoted by Detilleux et al. (1994) and Wanner et al. (1998). In sheep an estimate of 0.09 for the infection status assessed by bacteriological analyses was reported by Riggio et al. (2010) and Tolone et al. (2013) in the Valle del Belice breed. However, it was reported that with imperfect sensitivity and, particularly, specificity, the heritability of liability is likely to be substantially underestimated. In other words, there may truly be more genetic variation for the liability to mastitis than the field data suggests (Riggio

et al., 2010). Tolone et al. (2013) reported a genetic correlation between SCS and the infection status, as assessed by bacteriological analyses of 0.93, suggesting that selection for low SCS could also lead to a reduced incidence of mastitis. These results, therefore, indicate that selection for reduced SCS can help to reduce mastitis incidence. In this regard, results by Rupp et al. (2009) from a first-lactation survey in dairy sheep have provided evidence that selection based on SCS estimated breeding values (EBVs) may help to improve resistance to clinical and subclinical mastitis. Low SCS line animals showed a lower incidence of clinical mastitis, a lower prevalence of mammary abscesses and subclinical intramammary infections, especially at parturition. A better ability to recover from intramammary infections contracted during lactation and a lower SCS in bacteriologically positive samples was also found. These results were also emphasized by Riggio et al. (2010), suggesting that animals with a high SCS in bacteriologically negative samples, are more prone to mastitis. Therefore, the approach of selecting animals for decreased SCS is justified and should help to reduce the prevalence of mastitis, even in the absence of knowledge about infection status of the animal. This is in agreement with what previously reported in cattle. Philipsson et al. (1995) have estimated a linear relationship between SCC and the occurrence of clinical mastitis – concluding that the selection for lower SCC was desirable and that a lower level of SCC reflects a reduced incidence of infection, rather than a reduced ability to react to it. Moreover, Rupp et al. (2000) concluded that cows with the lowest mean SCC in the first lactation had the lowest risk for clinical mastitis in the second lactation. These results, therefore, suggest that breeding goals should favour animals with the lowest observed SCC. Nevertheless, it has been stated that by decreasing the milk SCC to very low levels by selection, could impair the animal's capacity to combat intramammary infection. Some of the milk resident cells, such as macrophages, are essential in initiating the inflammatory process in response to intramammary invading pathogens. Therefore, it might be useful to monitor if this (i.e.

280 selection for the lowest SCC level) does not affect the ability to resist infections. A better  
281 understanding of the defence mechanisms affected or modified by such a selection could be  
282 indeed helpful, to predict indirect responses on udder health in the long term and, if  
283 necessary, to modify the selection modality and criteria accordingly. It could also be  
284 important to monitor the actual mastitis incidence in the population by, for example,  
285 collecting information on the infection status at regular intervals to ensure that selection on  
286 correlated traits still results in the desired improvement of udder health.

287 When deciding upon the most appropriate trait to select for, one should also take into account  
288 the sociocultural background of the farmers. Compared to the collection of information on  
289 infection status or clinical mastitis, it is easier, cheaper, and less time-consuming for farmers  
290 to collect information on SCC. This can be regularly recorded during milk recording at a low  
291 cost. In this case, therefore, farmers would likely be more willing to cooperate because of the  
292 low costs and high frequency of recording. In contrast, samples for determining the infection  
293 status have to be collected with more care, than samples for SCC. The implementation of a  
294 protocol for collecting such samples by farmers may be difficult, requiring more commitment  
295 in order to ensure sufficient quality of sample collection. It may therefore also be necessary,  
296 in this case, to have these samples collected by more qualified persons, with the obvious  
297 disadvantages of higher costs and additional time by the farmers.

298 It is important to highlight, however, that in most of the sheep breeds, current selection is  
299 mainly practised on a “within farm” basis and based on the performance of the ewes. In this  
300 situation, according to the considerations drawn so far, it is unlikely that selection for mastitis  
301 resistance will be successful – independent of the use of infection status or SCS. Based on the  
302 above considerations, therefore, the implementation of a well-structured breeding programme  
303 needs to be realized, in order to guarantee reliable pedigree recording and performance

registration. At present, only a few dairy populations worldwide, mainly located in the Mediterranean region or in North America, have the required organization to allow the development of a large-scale recording and genetic evaluation (Rupp and Foucras, 2010). To current knowledge, the French Lacaune breed is the only small ruminant dairy breed selected for increased udder health (Rupp et al., 2002) – with genetic evaluations for the lactation mean SCS, run since 2002, based on a simplified recording system for SCC and implemented in the same way as that for milk fat and protein content (Rupp et al., 2002).

## ***5.2. Genetic correlations between SCS and other traits***

Although farmers select on several traits, based on own performance, milk yield is currently the most important selection criterion, for which phenotypic records are collected and breeding values are estimated, in most dairy sheep breeds. Barillet (1997) suggested that the introduction of milk composition traits and/or functional traits (e.g. resistance to mastitis) as selection objectives should be addressed only when a breeding programme has reached an asymptotic annual genetic gain for milk yield. However, this ignores the correlated response in other economically important traits, resulting from selection on milk production only. To quantify the likely correlated responses, it is important to determine the genetic correlations between different traits.

Unlike bovine mastitis, where the genetic antagonism between SCS and milk production traits is well documented, genetic correlation estimates between milk production and mastitis traits are quite inconsistent across dairy sheep studies. Published genetic correlations between SCS and milk yield range from positive i.e. antagonistic, to negative (Baro et al., 1994; El-Saied et al., 1998; El-Saied et al., 1999; Barillet et al., 2001; Rupp et al., 2003a; Riggio et al., 2007).

Another interesting aspect to consider is the correlation between SCS and udder conformation traits, which are favourable according to literature (Legarra and Ugarte, 2005; Sechi et al., 2007). Results suggest that udders with what is perceived to be a good shape are less affected by sub-clinical mastitis. Pendulous udders have been associated with an increase in SCC (Casu et al., 2010; Huntley et al., 2012). Pendulous and deep, poorly attached udders are difficult to milk and may cause sudden cluster falling, teat-end impacts, and subsequent bacterial infections (Bergonier et al., 2003). In addition, these udders are more prone to injuries (Legarra and Ugarte, 2005). However, this is a bit controversial, as Huntley et al. (2012) showed that teat lesions were not significantly associated with a change in udder half SCC, suggesting that teat lesions do not increase the risk of bacterial invasion of the udder.

## **6. Alternative statistical modelling for SCC/SCS**

In using SCC as an indicator of mastitis, the dynamic nature of mastitis is often ignored in the statistical analysis. It has been reported that both clinical and subclinical mastitis cause deviations from a typical curve of SCC (de Haas et al., 2004). In this respect, the use of individual SCC test-day records is an improvement, compared to the average of SCC records collected during a lactation. However, Urioste et al. (2010) reported that the use of test-day SCC can still make it difficult to identify short-duration infections, as SCC is often only recorded at approximately monthly intervals. Therefore, Urioste et al. (2010) suggested exploring alternative traits derived from the SCC curve (e.g. traits designed to capture SCC base levels and variation along the curve, time and level of infection, and time of recovery). Ideally, these alternative traits should be able to accommodate sudden and drastic changes in SCC, which in turn may improve the diagnosis of mastitis and hence increase genetic progress in mastitis resistance. There are, however, limitations to the use of these alternative traits on commercial farms. If it is true that the shortcoming of SCC is that it is only recorded



monthly, making it difficult to identify short-duration infections, then these alternative traits are unlikely to contain more information as they are based and designed on the same original information (i.e. test-day SCC). Moreover, ewes are milked (and, therefore, SCC records available) only once lambs are fully weaned, which could lead to an early misclassification of healthy and infected animals. Therefore, these alternative traits can probably be explored, used and better exploited on experimental farms, where the SCC records can be collected more frequently.

In the genetic evaluation of SCS, information collected on healthy (i.e. non-infected) and infected animals, is treated equally. However, several researchers suggested that, in cattle, SCS in healthy and infected animals are different traits (Detilleux and Leroy, 2000; Boettcher et al., 2007; Madsen et al., 2008). This was also confirmed in sheep by Riggio et al. (2010), who showed that SCS in healthy and infected animals can indeed be considered as different traits – with different heritabilities, and with a genetic correlation between bacteria negative and bacteria positive SCS of 0.62. Whilst this genetic correlation is moderately positive, it is significantly less than unity, suggesting that bacteria negative and bacteria positive SCS are not the same trait. The genetic evaluation of SCS can be improved when this non-unity genetic correlation is taken into account. In most countries, however, cases of mastitis are not routinely recorded in a systematic manner. The lack of information on the infection status is a limitation in selecting directly for mastitis resistance. It implies that when using SCS as an indicator of mastitis, no distinction can be made between SCS data from infected and non-infected animals.

When information on the infection status is not available, SCS may be regarded as a mixture of observations from animals with unknown health status, i.e. with and without mastitis. Mastitis infection would produce a deviation from the SCS baseline level, i.e. an observed

test-day SCS can be regarded as resulting from effects of a baseline SCS (a continuous trait) and a deviation caused by a binary process (healthy or infected status). Detilleux and Leroy (2000) have shown that a finite mixture model can account for these differences and can represent a latent structure in a set of data, whereby observations may belong to one of several distributions – possibly differing in mean, variance, and even the type of distribution (McLachlan and Peel, 2000). Recently, ten Napel et al. (2009) showed that there is indeed evidence in the distribution of SCC values that some SCC are an indication of an infected udder or quarter and others are indicative of a response to infection or a recovery from an infection. These researchers highlighted that by describing the observed distribution by a mixture of 4 normal and 1 exponential distributions provides an opportunity to distinguish between non-infected animals and animals infected with minor or major pathogens. Using mixture models, therefore, the selection for reduced mastitis incidence may be based on the probability of mastitis given SCS, rather than selection for lowest possible SCS. More recent research has also been done to extend the ideas of Detilleux and Leroy (2000) to develop a finite mixture model for SCS, using a Bayesian approach (Ødegård et al., 2003; Gianola et al., 2004; Boettcher et al., 2007). Boettcher et al. (2007) tested four different mixture models and all were found to be more appropriate for analysis of SCS data, than the standard linear model. Moreover, although correlations of ca. 0.90 were recorded between breeding values from the mixture and linear models, changes in ranking of the higher ranked sires were reported, showing that practical benefits would be realized with the adoption of a mixture model for genetic evaluation. However, it has to be highlighted that although mixture models are potentially useful and a good alternative for analysis of SCS data, they require good data recording. Moreover, these models may be difficult to implement in practical breeding values estimations, because of computational limitations.

## **7. Actual situation and prospects for improvement**

An accurate selection criterion must be a relevant biological trait genetically well correlated to mastitis resistance, exhibit sufficient genetic variability and have operational properties, such as easy and cheap measuring procedure on a large scale. Based on these considerations, SCC is the most widely used criterion to achieve better udder health. Repeated SCC data are indeed routinely recorded for individuals as part of milk recording schemes. Nevertheless, it is important to keep in mind that the genetic response will always be limited – as breeding objectives still favour milk quantity and content from an economic point of view.

In setting up a breeding programme, however, there are other issues that are important to take into account. Technical and infrastructural related issues, for example, are the greatest bottlenecks in genetic improvement programmes for most of the sheep farming systems. Small flock sizes, poor pedigree and performance recording, lack of clear breeding goals, lack of or poor infrastructures. These are all factors that contribute to the low participation of farmers in breeding schemes, which in turn makes achieving within-breed genetic improvement highly challenging.

Whereas artificial insemination (AI) is a common reproductive technique in dairy cattle, in dairy sheep its application is limited to experimental farms. Due to the low use of AI, the diffusion rate of a ram is from 100 to 1000 times lower than that of a bull (Carta et al., 2009). The limited use of AI, therefore, reduces the progeny group size of rams and is in general associated with poor pedigree recording, which negatively affects the accuracy of breeding value estimates (Van Vleck, 1970; Lee and Pollak, 1997). Many flocks rely on a few males, and it is not possible to know with certainty which ram is the sire of an animal. In dairy cattle, it has been reported that paternity errors can reach up to 20% of registered animals (Ron et al., 1996) and this percentage is probably even higher in sheep, drastically reducing

426 the genetic gain and the success of breeding programmes. To overcome this problem, it is  
427 possible for farmers to manage natural mating by grouping ewes with a single ram (i.e.,  
428 mating group) during the mating period. This management strategy would make it easier to  
429 determine the correct sire of a lamb, based on the lambing date. However, the poor  
430 infrastructures on the farms in general do not allow for the implementation of these strategies.  
431 As an alternative, it may be possible to use DNA testing for pedigree verification or pedigree  
432 assignment in cases of unrecorded mating or the use of multiple sires. Procedures have been  
433 already developed for both goats and sheep (Glowatzki-Mullis et al., 2007; Rosa et al., 2013),  
434 as well as dogs (DeNise et al., 2004), horses (Tozaki et al., 2001; Seyedabadi et al., 2006),  
435 and cattle (Van Eenennaam et al., 2007).

436 Another problem encountered in genetic evaluation of sheep flocks is the poor genetic  
437 connections between flocks, which result from the limited exchange of rams between farms.  
438 This could be overcome by AI, but as discussed earlier the uptake of AI is low. This implies  
439 that improvements in genetic connections need to come from exchanging rams between  
440 farms. However, farmers do not see it as favourable to exchange rams between flocks, as they  
441 usually think they have the best individuals. An alternative would be to implement a selection  
442 scheme based on the pyramid management of the population, which is nowadays considered  
443 the most efficient selection scheme for local dairy sheep (Barillet, 1997). In this scheme, the  
444 nucleus flocks are at the top of the breeding pyramid. In these flocks, pedigree and milk  
445 recording are implemented, and breeding value estimations are carried out to generate genetic  
446 progress in these flocks. The genetic progress would be then disseminated to commercial  
447 flocks through AI or natural-mating rams originated from nucleus flocks. A potential problem  
448 in the implementation of this scheme is that farmers would need to be convinced regarding  
449 the superior quality of the rams from the nucleus flock. However, it is likely that farmers will

be willing to cooperate in such a scheme once they experience the quality of the breeding products. It would even be easier to realize such a scheme if it were technically or financially supported by the Government, Breeder Associations or the University. The support by such an Institution would reassure farmers, who sometimes just need to feel that their interests are taken into account.

When implementing a nucleus breeding scheme, an important aspect is the genotype by environment (GxE) interaction. GxE interactions could reduce the benefits for commercial farmers of genetic progress generated in the nucleus flock. One of the methods used to quantify GxE, is the estimation of genetic correlations ( $r_g$ ) between traits measured in different environments. When  $r_g$  between the phenotypic values of the same trait expressed in different environments is high i.e. equal or close to 1 – then there is no GxE (Robertson, 1959). On the other hand, low  $r_g$  values indicate GxE, i.e. phenotypes expressed in different environments are expressions of different traits. Mulder and Bijma (2005) estimated that a  $r_g$  of 0.80 between two environments results in 20% less genetic gain for a trait in dairy cattle, when breeding stock are selected in another environment. Mulder et al. (2006) demonstrated that in dairy cattle, when  $r_g$  between environments are between 0.50 to 0.70, a single breeding programme with progeny testing bulls in different environments would be optimal to breed for general adaptability. However, when  $r_g$  between environments is lower than 0.50, environment-specific breeding programmes are necessary to breed for specific adaptability. Therefore, to realize a pyramid selection scheme for any breed, it would be important to make sure that the environment of the nucleus flocks is comparable to that at the commercial farms.

Concerning diseases and disease resistance, quantifying and accounting for the impact of environmental factors is an important part of identifying and measuring true host genetic

variation in resistance to the disease under study. There is a risk of bias in genetic parameter estimates and lost opportunities in identifying individuals with extreme genetic risk, when these environmental factors are not correctly taken into account (Bishop and Woolliams, 2010). It is therefore necessary to determine the “optimal exposure level” in order to select for mastitis resistance. Of course it would not be good to have all animals being infected; however, on the other hand, if no animals are affected then there is no information upon which to base the selection. It is important to realize that a lack of exposure simply means that individuals do not have the opportunity to express their genetic merit for resistance, with potentially highly susceptible individuals being (wrongly) classified as resistant, simply because they are healthy (Bishop and Woolliams, 2010). These researchers have also demonstrated that whilst true presence/absence of a disease, given exposure to infection, is largely a function of the immune response, the actual prevalence of the disease and the estimable genetic variation between animals will be influenced by variable exposure and the sensitivity of diagnosis.

In implementing a breeding scheme for mastitis resistance, it has to be taken into account that measurements of phenotypic indicators for mastitis resistance are time and labour intensive. Therefore, the use of genetic markers to indicate resistance or susceptibility to mastitis or to better exploit the phenotypic information through genomic selection (GS) is an attractive proposition (Goddard and Hayes, 2007). At present, however, the available literature on GS and molecular markers for mastitis resistance mainly refer to dairy cattle (Klungland et al., 2001; Boichard et al., 2003; Schulman et al., 2004). In sheep, quantitative trait loci (QTL) influencing SCS have recently been detected (Rupp et al., 2003b; Gutierrez-Gil et al., 2007; Raadsma et al., 2009).

There is currently widespread excitement regarding the potential for GS to provide new approaches for the improvement of sustainability traits in Holstein dairy cows. Many breeding programmes worldwide have already implemented GS. However, it is important to recognize that it is not obvious how GS can be implemented in small ruminant species. An important limitation of applying GS to sheep, is that a reference population of considerable size would be required. In dairy cattle, for example, reference populations of over 4000 progeny tested young bulls are available, and this scale would be difficult to achieve in sheep. However, nowadays, thanks to the development of high-density SNP arrays with tens of thousands of genetic markers spread across the genome, research is moving to the direction of GS in sheep as well, as such arrays have also proven to be very powerful, with even a small number of animals. In a GS study conducted on the Lacaune breed on three traits (milk yield, fat content, SCS), Duchemin et al. (2012) have demonstrated that molecular markers can be effectively used to improve current selection methods. Using a reference population of about 2500 proven rams and about 44000 SNP, it was reported that accuracies of GEBV for males at birth can be improved from +18 to +25%, according to the traits.

## **8. Conclusions**

Although results reported in the literature for sheep are less frequent than for cattle, it seems to be accepted that selection for reduced SCS would lead to a reduced mastitis incidence. This review, however, highlights a number of elements that need to be considered when setting up a breeding programme for mastitis resistance, using SCS as an indicator. Besides the importance of knowledge of both genetic and environmental aspects of the traits considered, the need has been stressed for having a strong and well-structured organization to implement and support the programme. The heritabilities of the traits of interest, either SCS or infection status, are indeed low. Therefore, it is unlikely that selection for mastitis

resistance by the farmers on their own will be successful. However there is a good prospect for genetic improvement at farm level, when reliable pedigree and performance recording is implemented across flocks and combined with breeding value estimation. This system requires cooperation between the farmers and technical support from an independent organisation.

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